

**Project Report: Continued surveillance and characterization of pepper resistance-breaking strains of *Tomato spotted wilt virus* (TSWV) in the Central Valley of California and screening for sources of resistance**

**Project Leader: Robert L. Gilbertson**, Distinguished Professor  
Department of Plant Pathology, University of California-Davis (UC Davis)  
1 Shields Ave. Davis, CA 95616,  
Phone: 530-752-3163 FAX: 530-752-5674,  
e-mail: [rlgilbertson@ucdavis.edu](mailto:rlgilbertson@ucdavis.edu)

**Maria Rojas**, Project Scientist  
Department of Plant Pathology UC Davis  
email: [mrrojas@ucdavis.edu](mailto:mrrojas@ucdavis.edu)

**Margaret Cespedes**, Laboratory Assistant  
Department of Plant Pathology, UC Davis  
email: [eccespedes@ucdavis.edu](mailto:eccespedes@ucdavis.edu)

**Matthew Terra and Amelia Teicheira**, Eckert Co. and **members of our Farm Advisor/PCA Network**

## **Background**

Numerous plants viruses can infect and cause disease symptoms and, sometimes, cause substantial economic losses. The virus that poses the great threat in California pepper production is tomato spotted wilt virus (TSWV), which is transmitted by the western flower thrips (*Frankliniella occidentalis*). This insect pest is difficult to control and also can cause direct damage to peppers. Peppers in California can also be infected by alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV) and several potyviruses, which are vectored by aphids and beet curly top virus (BCTV) that is vectored by the beet leafhopper. Furthermore, curly top disease caused by BCTV is emerging as a more important disease in California, appearing in new crops and locations and being caused by new strains. Finally, there are a number of economically important pepper-infecting viruses that occur in Mexico that could be introduced into California, e.g., the tomato brown rugose fruit virus (ToBRFV) and whitefly-transmitted begomoviruses, such as pepper Huasteco yellow vein virus (PHYVV). Because rapid detection is critical to effective management, we believe that annual virus surveillance is the best way to do this and we have the capacity to recognize and test for these viruses.

Spotted wilt is most effectively managed with an IPM approach, which often involves the planting of resistant pepper varieties that possess the *Tsw* gene, a single dominant gene introgressed from *Capsicum chilense*. TSWV also causes spotted wilt in tomato, where a different single dominant gene, *Sw5*, was used to generate resistant tomato varieties. A problem with single dominant (strong) resistance genes like *Tsw* and

*Sw5* is the selection for TSWV strains that can overcome (break) this resistance. This was the case in 2016, when a California tomato RB-TSWV strain appeared, and overcame the *Sw-5* resistance gene in tomato in Fresno County. This tomato RB-strain infects and causes spotted wilt symptoms in pepper, but does not break the *Tsw* gene (i.e., did not infect cv. Huntington [Tsw+]). This California tomato RB TSWV strain is now prevalent in Fresno, Kings and Merced Counties and was detected in Northern Counties in 2021 and 2022.

With the increasing cultivation of varieties with the *Tsw* gene in California, the identified pepper RB TSWV strains in Yolo County in October 2017 was not unexpected. This virus isolate (RB-TSWV-CA-P-1) came from a mature red fruit of a Tsw variety that showed ringspots and blotches, typical of spotted wilt. Following mechanical inoculation of cv. Huntington (the standard test for pepper RB TSWV), this isolate caused severe spotted wilt symptoms. Moreover, the continued detection of pepper RB TSWV strains in multiple locations in California (Fresno, Merced, San Joaquin, Santa Clara and Yolo Counties) in 2018 and 2019 indicates that pepper RB TSWV has been persisting. Thus far, these pepper RB strains appear late in the season, but can cause economic loss in mature red peppers in late-planted fields.

Here, it is important to point out that the California tomato RB-TSWV strain that appeared in 2016 and breaks the *Sw-5* gene of tomato, infects and causes spotted wilt in pepper, but does not break the *Tsw* gene (i.e., does not infect cv. Huntington). Conversely, the pepper RB TSWV isolated from Tsw pepper fruit in 2017 (e.g., RB-TSWV-CA-P-1) does infect tomato plants, but does not break the *Sw-5* gene.

**Table 1. Major resistance-breaking (RB) tomato spotted wilt strains in California and their infection of differential species/varieties**

	Pepper	Pepper	Tomato	Tomato	Tobacco
TSWV strain	Susceptible	Resistant <i>Tsw</i> gene	Susceptible	Resistant <i>Sw-5</i> gene	<i>N. benthamiana</i>
Wild-type	+	-	+	-	+
Pepper RB	+	+	+	-	+
Tomato RB	+	-	+	+	+
Super RB	+	+	+	+	+

However, there are notable difference between the emergence of pepper RB-TSWV and tomato RB-TSWV strains in Central California. First, the tomato RB-TSWV strain has become the predominant strain infecting processing tomatoes in Central California, whereas pepper RB-TSWV typically appears late in the season and to date it has not emerged as the predominant strain of TSWV infecting peppers in California. Second, resistance to TSWV in pepper and tomato is conferred by two different resistance genes. Third, the viral gene product or effector that is recognized by these TSWV resistance genes is different: the *Tsw* gene of pepper recognizes the NSs protein, a viral

suppressor of gene silencing, and *Sw-5* gene recognizes the NSm protein, which is the viral movement protein. Finally, the NSm protein of the tomato RB-TSWV has a tyrosine (Y) amino acid residue at position 118 (versus a cysteine [C] in the wild-type protein), which is a marker for tomato RB-TSWV strains. Furthermore, the mutation in the viral RNA sequence was used to design a RT-PCR test specific for this tomato RB-TSWV strain. To date, no such marker has been identified in the NSs protein of pepper RB-TSWV strains, and there is currently no molecular test for pepper RB TSWV strains.

In 2019 we identified a super RB (SRB) strain that breaks the resistance of the *Tsw* gene in pepper and the *Sw-5* gene in tomato (Table 1). This strains has been detected in Fresno and Yolo Counties. If this strain builds-up in processing tomato, it could emerge as a more important problem for pepper production. Therefore, it is important to assess the potential for these pepper RB TSWV strains becoming a greater economic problem and to identify sources of resistance to RB strains.

### Overall Objectives:

The overall objectives of this project are to 1) identification and management of viruses that pose a threat to pepper production in California, and 2) understand the biology and threat posed by pepper RB strains of TSWV, especially the supper RB strain.

### Specific Objectives for 2022:

#### 1. Virus surveillance in peppers in 2022 with emphasis on RB TSWV strains

**Curly top.** The overall level of curly top disease caused by BCTV in California pepper crops was low in 2022. A single cv. Huntington plant with curly top symptoms from Merced was received 15 June and results of PCR tests confirmed infection with the mild-type BCTV strain, BCTV-Colorado (CO). In mid-July, samples from pepper plants with curly top symptoms from another field in Merced were received, and all seven were positive for BCTV infection with the multiplex PCR tests (Table 2). Typing of strains revealed that 5 plants were infected with the common BCTV-CO strain and one with a severe-type strain, BCTV-LH71, known to occur in the area. What was not expected was the detection of the emergent BCTV-Spinich curly top (SpCT) strain that has been involved in the curly top outbreaks in the Northern Counties in 2021 and 2022.

**Table 2. Results of tests of pepper samples with curly top symptoms in 2022**

Sample	Location	Multiplex PCR	BCTV strains detected
22-442	Merced	+	Severe (SpCT)
22-443	Merced	+	Mild (CO)
22-444	Merced	+	Mild (CO)
22-445	Merced	+	Mix (SpCT and CO)
22-446	Merced	+	Severe (LH71)
22-447	Merced	+	Mild (CO)
22-448	Merced	+	Mild (CO)

**Table 3. Results of tests of samples with virus-like symptoms and recovered TSWV isolates from pepper in Merced and a ‘hotspot’ area for RB-TSWV in Gilroy**

Sample	Location	R/S	Analyses performed in the lab				
			Immuno-strip	Tsw gene	RT-PCR Tomato RB	RT-PCR NSs	Reaction on pepper cv. Huntington
TS 22-318	Merced-6-15	S	+	NT	+	+	RB
TS 22-554	Gilroy-8-4	S	-	NT	NT	NT	NT
TS 22-555	Gilroy	S	-	NT	NT	NT	NT
TS 22-556	Gilroy	S	+	NT	-	+	RB
TS 22-557	Gilroy	S	+	NT	-	+	**
TS 22-558	Gilroy	S	-	NT	NT	NT	NT
TS 22-559	Gilroy	S	NT	NT	+	+	RB
TS 22-560	Gilroy	S	NT	NT	+	+	RB
TS 22-561	Gilroy	S	NT	NT	-	+	RB
TS 22-586	Gilroy-8-18	R	NT	-	+	+	NT*
TS 22-587	Gilroy	R	NT	-	+	+	NT*
TS 22-588	Gilroy	R	+	-	+	+	RB
TS 22-589	Gilroy	R	+	-	-	+	RB
TS 22-590	Gilroy	R	NT	-	+	+	RB
TS 22-591	Gilroy	R	+	+	+	+	RB
TS 22-592	Gilroy	R	NT	-	+	+	RB
TS 22-593	Gilroy	R	NT	-	+	+	RB
TS 22-594	Gilroy	R	NT	-	+	+	RB
TS 22-595	Gilroy	R	NT	-	+	+	RB
TS 22-596	Gilroy	R	NT	-	-	+	**
TS 22-597	Gilroy	R	NT	-	+	+	**

NT: No tested

R/S=fields planted with a resistant (Tsw+) or susceptible (Tsw-) variety

\*Mixed infection with tobamovirus.

\*\*These isolates did not infect *Nicotiana benthamiana*

**Spotted wilt.** In 2022, a relatively small number of samples were received for testing for spotted wilt in peppers. Moreover, because our surveillance cooperators from Eckert were surveying pepper fields at locations statewide and our Farm Advisor/PCA Network was connected with other growers, we conclude that the overall incidence of spotted wilt in peppers in 2022 was relatively low, especially in resistant varieties.

**A potential hotspot for SRB-TSWV in Gilroy.** In 2022, Bob Heisey visited an area of Gilroy where resistance-breaking strains of TSWV were recovered more than 15 years ago by Dr. Jim Moyer’s group at North Carolina State University. Samples were collected from two pepper fields, one planted with a susceptible (S) cultivar and the other with the resistant cv. Huntington. The samples from the field with the susceptible variety had symptoms including mosaic and yellowing to necrosis, ringspots and yellowing, typical of TSWV infection. The plants with the TSWV symptoms (5/8) were confirmed infected with TSWV. Four of these TSWV isolates were pepper RB strains based on capacity to infect cv. Huntington plants, and two of these (TS-222-559 and -

660) were positive in the RT-PCR test for tomato RB-TSWV, indicating these are SRB strains.

The samples received 18 August were from a field planted with cv. Huntington and included 12 foliar and fruit samples with typical TSWV symptoms (Table 3). TSWV infection was confirmed by RT-PCR with the NSs primer pair, and 10 of these were tomato RB strains (Table 3). Eight isolates were inoculated onto cv. Huntington plants and all were pepper RB-TSWV. Thus, these eight isolates are SRB TSWV strains.

Taken together, these results show that regardless of the variety, all of the TSWV isolates from this area of Gilroy were pepper RB and most were also tomato RB, indicating these are SRB TSWV strains. It should be noted that the *Tsw* gene was not detected in most of the samples, suggesting these were self-pollinated or incomplete hybrids. These results support the hypothesis that this area is a 'hotspot' for RB TSWV strains, and suggests these may be the indigenous strains in this area.

## ***2. Monitor fields in Merced where outbreaks of SRB TSWV occurred in 2021.***

A sample of a banana pepper plant with spotted wilt symptoms, especially in the fruit, was received 15 June from Merced. TSWV infection was confirmed and RT-PCR indicated this was a tomato RB strain (Table 3). Furthermore, this isolate (TS-22-318) infected cv. Huntington, indicating that it was a SRB strain. This showed that the SRB strain persisted in Merced and emerged early in the growing season. Fortunately, this isolate (and spotted wilt) did not spread substantially or cause economic loss in early- or late-planted peppers in Merced in 2022.

### **Key takeaways from 2022 research:**

- 1. There was an overall low incidence of pepper virus diseases in 2022**
- 2. Curly top was detected in two fields early season (June and July) but at low incidence**
- 3. The emergent BCTV-SpCT strain was detected in peppers with curly top symptoms**
- 4. A potential hot-spot of SRB-TSWV in Gilroy may have been revealed**
- 5. Late-season outbreaks of pepper RB-TSWV were not observed in 2022**