Project Report: Virus surveillance in peppers for resistance-breaking (RB) strains of tomato spotted wilt virus (TSWV) and assessment of the susceptibility of peppers to emerging tomato-infecting tobamoviruses

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Special thanks:
Max Babylon and Jeanmarie Harty, Bayer

Overall objective:

To (i) provide an effective statewide surveillance system for virus (and other) diseases of peppers in California, (ii) specifically monitor and research exotic and newly emerged viruses as well as unusual outbreaks of known viruses, and (iii) provide outreach in the form of field visits, grower talks, and detailed reports and blogs

Please note that consistent with this overall objective, we focused our effort, time and resources on investigating the unusual outbreak of curly top disease (CTD) in peppers in Ventura in 2024 and, thus, this became our specific objective 2.

Specific Objectives:

1. Virus surveillance in peppers in 2024 with emphasis on RB TSWV strains.

As in previous years, we rely on receiving samples from our network of (i) Farm Advisors (ii) growers and PCAs, (iii) Matt Terra and his team as they visit fields and trials statewide, and (vi) our own fied visits and surveys. We believes this provides us a high probability of being aware of virus disease outbreaks in peppers in California.

Low incidence of virus disease in pepper crops in California in 2024. In 2024, the incidence of virus diseases of pepper was very low. As is shown in Table 1, we detected alfalfa mosaic virus (AMV) in a small number of samples from Yolo (3) and Ventura (2) and cucumber mosaic virus (CMV) in a sample from Fresno County. These aphid-transmitted viruses are common in pepper and plants often recover from infection with AMV.

Table 1. Total number of samples collected or received from pepper fields and results of tests for different viruses.

County	Total samples	TSWV						Other	
		Total	TSWV-RB tomato				TSWV-RB pepper[b]	AMV	CMV
			Negative	CPN	YPT	Mix			
Yolo	22	19	1	3	8	7	7/11	3	0
Fresno	1	0	---[a]	---	---	---	---	0	1
Ventura	2	0	---	---	---	---	---	2	0

[a], not tested

[b], number of samples infecting pepper cv. Huntington/number of inoculated samples (only 11 isolates were recovered in *N. benthamiana*).

Pepper RB TSWV detected in Yolo County in 2024 but at low levels. In terms of spotted wilt, we did not receive samples in 2024, and we were not aware of outbreaks of spotted wilt in resistant varieties in commercial fields. We collected 19 samples from plants of resistant varieties with spotted wilt symptoms in two fields in Yolo County in 2024. RT-PCR tests indicated that the TSWV infecting all but one of these samples possessed tomato RB TSWV genes, i.e., CPN (new RB strain from Sutter/Colusa) or YPT and sometimes in mixed infections (Table 1). This indicates that most of the TSWV in

these fields was tomato RB, consistent with our results with samples of spotted wilt from monitored processing tomato fields. Furthermore, 11 isolates were inoculated onto susceptible and resistant peppers and 7 infected both the susceptible (Cal Wonder) and resistant (cv. Huntington) plants, confirming that these are pepper RB strains. Furthermore, because these are also tomato RB positive, they are isolates of the new super-RB (SRB) strain (Table 2).

Notably, in contrast to 2023 when levels of spotted wilt in processing tomato fields in the Northern production area were relatively high, levels in 2024 were relatively low. Although this difference could reflect different weather conditions, both years were cosidered relatively cool and wet. Another possible explanation comes from results of a rapid molecular identification method for insects based on the DNA sequence of the cytochrome oxidase gene I (COTI) gene, which indicated that a substantial portion of the thrips captured on yellow sticky cards in the Northern production area were onion thrips (*Thrips tabaci*), which prefer to feed on members of the onion family and are not good vectors of TSWV. These are preliminary results, but suggest that the composition of the thrips population may impact TSWV spread, at least in the Northern production area.

Table 2. 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 *Tsw* gene**	**Susceptible**	**Resistant *Sw-5* gene**	***N. benthamiana***
Wild-type	+	-	+	-	+
RB pepper	+	+	+	-	+
RB tomato	+	-	+	+	+
Super RB	+	+	+	+	+

2. Identification of an outbreak of curly top disease in peppers in Ventura Co in 2024

Around the middle of July, we received photos and samples of peppers with virus-like disease symptoms including stunting, up curled and yellow leaves and small distorted fruits from fields in Ventura County (Piri area) from Max Babylon and Jeanmarie Harty of Bayer (Figure 1). These symptoms looked like those of CTD caused in pepper by beet curly top virus (BCTV). There also were pepper plants that showed milder virus-like symptoms of light green to yellow mosaic and leaf crumpling, more typical of a mosaic-type virus. However, immunostrip tests of leaves with these mild symptoms for typical mosaic viruses, i.e., AMV, CMV, TSWV and general potyvirus were all negative.

We next tested leaf and fruit (often have high levels of BCTV) samples from plants with the typical symptoms of CTD for BCTV infection with a multi-plex PCR test that indicates if the samples is infected with BCTV and whether it is a ‘mild’ or ‘severe’ type BCTV strain based on the size of the PCR-amplified fragment (Figure 2). As shown in Table 3 and Figure 2, 10/17 samples were clearly positive for BCTV infection (lanes where a DNA fragment is amplified), and all were mild-type isolates.

Figure 1. Curly top disease (CTD)-like symptoms of small distorted fruit at the top of a pepper plant from Ventura County (Piru area) received 7/19/24

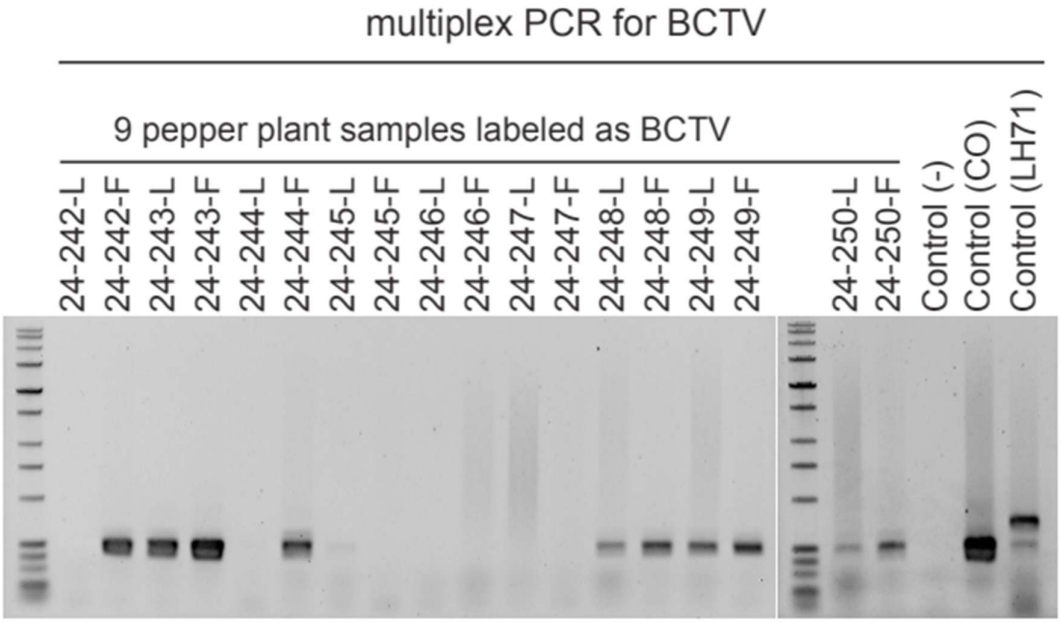
Furthermore, tests of these positive samples with strain-specific BCTV PCR revealed all 10 were BCTV-Worland, which is one of the strains known to infect pepper and it is known to occur in Ventura County. Thus, these results confirmed that the CTD-like symptoms were indeed CTD of pepper and were caused by BCTV-Wor

Table 3. Total number of samples collected or received from pepper fields and results of tests for beet curly top virus (BCTV) detection.

			BCTV					
			Multiplex PCR		Strain-specific PCR			
County	Total samples	Total samples tested[a]	Mild-type	Severe-type	CO	Wor	Mix	Negative
Ventura	19	17	10	0	0	10	1	6

[a], three samples were in bad condition and they were not tested. Also, one sample 24-191 had mixed infection of BCTV-Wor and AMV.

Figure 2. BCTV detection in samples of pepper plants with curly top-like disease symptoms received 7/19/2024 from Ventura County (Piru area)



Having failed to detect mosaic-type viruses infecting the pepper plants with the milder mosaic and crumpling symptoms, we next decided to test samples from these plants for BCTV infection. Here, the multi-plex PCR test was again used. Somewhat unexpectedly, these samples were positive for BCTV infection, including some with very low levels of virus (Figure 3). This indicates that these mild symptoms are due to BCTV infection. The low level of virus indicates that the plant may be less susceptible, which could be age-related or reflect some degree of genetic resistance. Thus, it will be of interest to investigate this phenomenon further.

Figure 3. BCTV detection in samples of pepper plants with mild symptoms of green yellow mosaic and crumpling of leaves received 7/19/2024 from Ventura County (Piru area)

Take home messages:

-Overall low incidence of virus disease in peppers in 2024

-Associated with cool wet winter/spring conditions (?)

-Pepper RB TSWV isolates detected in Yolo County in 2024

-Most were super-RB

-Low spotted wilt incidence in the Northern production area
associated with a high population of onion thrips (?)

-Pepper plants with stunting and yellow up-curled leaves
and small deformed fruits in Ventura Co. (Piru area) had CTD and
were infected were infected with BCTV-Wor

-Importance of virus and vector surveillance